2-17-95

MRID No. 429024-04

DATA EVALUATION RECORD

1. **CHEMICAL:** Oxine Copper.
Shaughnessey No. 024002.

2. **TEST MATERIAL:** 1) Quinolate 98; oxine copper or copper 8-quinolinolate; Batch No. 52390; 100% active ingredient; a green powder. 2) ^{14}C-oxine copper; Lot No. 041H9267; specific activity of 63.78 µCi/mg; 98% active ingredient.

3. **STUDY TYPE:** 123-2. Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: *Nitzschia punctata*.

4. **CITATION:** Ward, G.S. 1993. Oxine Copper (Copper 8-quinolinolate): Toxicity to the Saltwater Alga, *Nitzschia punctata*, Under Static Test Conditions. Laboratory Project ID No. J9006014m. Conducted by Toxikon Environmental Sciences, Jupiter, FL. Submitted by LA QUINOLEINE et ses dérivés, S.A., Paris, France. EPA MRID No. 429024-04.

5. **REVIEWED BY:**

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7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for a Tier 2 non-target aquatic plant study. The NOEC was not determined. Based on mean measured concentrations, the 5-day LOEC and EC_{50} for *N. punctata* exposed to oxine copper were 7.2 and 7.3 µg ai/l, respectively.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Species:** The *Nitzschia punctata* culture used in the test came from laboratory stock cultures originally obtained from the University of Texas, Austin. Stock cultures were maintained in saltwater algal medium under 4.6-6.5 klux continuous illumination, at a temperature of 23 ±1°C. The culture used as inoculum in this test was six days old.

B. **Test System:** Test vessels used were sterile 250-ml glass flasks which were capped. The test medium was the same as that used for culturing with the pH adjusted to 8.0 and filter sterilized (0.22 µm). The salinity of the medium was 30 parts per thousand (ppt).

One-hundred milliliters of the appropriate test or control solution were placed into each flask. The test vessels were kept in a growth chamber which illuminated the vessels with fluorescent light for 16 hours a day at an intensity of 4.2-4.9 klux. The temperature was maintained at 20 ±2°C.

A primary stock solution containing 14% radiolabeled material and 86% unlabeled material was prepared in acidified dimethylformamide (DMF). The concentration of oxine copper in the primary stock solution was 1 mg active ingredient (ai)/ml. Secondary (0.5 mg ai/ml) and tertiary (0.1 mg ai/l) stock solutions were prepared in DMF by serial dilution of the primary stock solution. Appropriate amounts of stock solution were added to nutrient solution (400 ml) to create the treatment solutions. All treatment solutions contained the same amount of DMF (0.1 ml/l).

C. **Dosage:** Five-day growth and reproduction test. Based on the results of a preliminary test, six nominal concentrations of 7.7, 12.9, 21.5, 35.7, 61.4, and 102 µg ai/l were selected for the definitive test. A solvent (0.1 ml DMF/l) and a medium control were also prepared.

D. **Test Design:** Each treatment and control was replicated three times. An inoculum of *N. punctata* cells was added to each flask that resulted in a cellular density of 10,000 cells/ml. The inoculum volume was 1.3 ml per flask. The flasks were randomly positioned in the chamber and were randomly rearranged daily after

observation. The solutions were hand-shaken twice daily. Cellular counts were performed daily by direct microscopic examination using a hemacytometer. The terminal counts were performed after 5 to 10 minutes of sonication. After 5 days of exposure, composite samples of the three replicates of the three highest concentration treatment solutions and of the control were added to fresh media and incubated for 9 days to determine whether the effects of the test material were algistatic or algicidal. The cells were enumerated on days 0, 2, 5, and 9.

The pH was measured at test initiation and termination in all control and treatment solutions. The temperature in the chamber was recorded continually and the temperature in an uninoculated flask was measured daily. Light intensity was also measured daily.

Samples were taken at test initiation and termination for analysis of the test material by liquid scintillation counting.

E. **Statistics:** All calculations were based on mean measured concentrations. Comparisons and percent effect values were determined against the pooled control. The 5-day EC values and associated 95% confidence intervals (C.I.) were computed using probit analysis. The no-observed-effect concentration (NOEC) was determined using analysis of variance and Dunnett's test ($p \leq 0.05$).

12. **REPORTED RESULTS:** Mean measured concentrations ranged from 89 to 98% of nominal (Table 1, attached). The mean measured concentrations were 7.19, 12.0, 20.9, 34.9, 54.3, and 91.7 µg ai/l.

Day-5 cell counts and percentage reduction in comparison to the pooled control for each concentration are presented in Table 2 (attached). Percent inhibition increased with increasing toxicant concentration and ranged from 40 to 100% inhibition.

Resuspension of the three highest concentration-exposed cells and control cells for nine days indicated that the test material was algistatic at a concentration of 34.9 and 54.3 µg ai/l and algicidal at a concentration of 91.7 µg ai/l.

Based on day-5 cell counts, the EC_{50} was determined to be 7.26 µg ai/l with a 95% C.I. of 5.14-9.10 µg ai/l. The NOEC

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could not be determined (<7.19 µg ai/l) due to a significant reduction in cell number at all treatment levels.

The pH was 8.0 in all treatment and control solutions at test initiation and ranged from 8.4 to 9.1 at test termination. Temperature ranged from 21.1 to 23.3°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were made by the author.

Good Laboratory Practice and Quality Assurance statements were included in the report indicating compliance with EPA Good Laboratory Practice Standards, 40 CFR Part 160.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedures were generally in accordance with the SEP and Subdivision J guidelines; the following are deviations:

The light intensity during the test (4.2-4.9 klux) was slightly higher than recommended (4 klux).

The test temperature (21.1-23.3°C) was greater than recommended (20°C).

The results of the continuous temperature measurements were not reported.

The type of lighting was not specified. Cool-white lighting is recommended.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to verify the EC value and Williams' test to verify the NOEC as well as determine the lowest-observed-effect concentration (LOEC). The reviewer obtained a slightly less conservative estimate of the EC_{50} (see attached printouts). The NOEC could not be determined and the LOEC was 7.2 µg ai/l.

C. **Discussion/Results:** Upon review of the algistatic portion of the study, the test material appeared to be algicidal at a concentration of 54.3 µg ai/l as well as 91.7 µg ai/l (Table 4, attached).

This study is scientifically sound but does not meet the guideline requirements for a Tier 2 non-target aquatic plant study. Based on mean measured concentrations, the 5-day LOEC and EC_{50} for *N. punctata*

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